

AMENDMENT

IN THE CLAIMS

Please amend the claims as follows, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents:

1. (Currently Amended) A chaperone polypeptide having refolding activity and having an amino acid sequence selected from at least amino acid residues 230-271 but no more than residues 150-455 or 151-456 of a GroEL sequence as shown in Figure 7 (SEQ ID NO 10), ~~wherein positions 262 and 267 are occupied by amino acid residues other than alanine and isoleucine, respectively,~~ or a corresponding sequence of a chaperone polypeptide, said corresponding sequence sharing at least 50% homology with said amino acid sequence, wherein position 262 of said amino acid sequence or said corresponding sequence, is occupied by an amino acid residue other than alanine, and wherein position 267 of said amino acid sequence or said corresponding sequence, is occupied by an amino acid residue other than isoleucine.

2 – 8. (Cancelled)

9. (Previously Amended) A polypeptide as claimed in claim 1, wherein the chaperone activity is determined by the refolding of cyclophilin A.

10. (Original) A polypeptide as claimed in claim 9 wherein 8M urea denatured cyclophilin A (100 μ M) is diluted into 100mM potassium phosphate buffer pH7.0, 10mM DTT to a final concentration of 1 μ M and then contacted with at least 1 μ M of said polypeptide at 25°C for at least 5 min, the resultant cyclophilin A activity being assayed by the method of Fischer G *et al* (1984) Biomed Biochim Acta 43: 1 101 -1111.

11. (Previously Amended) A polypeptide as claimed in claim 1 being an hsp60 polypeptide, or a GroEL polypeptide.

12. (Currently Amended) A polypeptide as claimed in claim 1 which comprises at least an amino acid sequence selected from GroEL residues:

- (a) 191-329, 191-330, 191-331, 191-332, 191-333, 191-334, 191-335, 191-336, 191-337, 191-338, 191-339, 191-340, 191-341, 191-342, 191-343, 191-344, 191-345, 191-346, 191-347, 191-348, 191-349, 191-350, 191-351, 191-352, 191-353, 191-354, 191-355, 191-356, 191-357, 191-358, 191-359, 191-360, 191-361, 191-362, 191-363, 191-364, 191-365, 191-366, 191-367, 191-368, 191-369, 191-370, 191-371, 191-372, 191-373, 191-374, 191-375 or 191-376, or
- (b) 192-329, 192-330, 192-331, 192-332, 192-333, 192-334, 192-335, 192-336, 192-337, 192-338, 192-339, 192-340, 192-341, 192-342, 192-343, 192-344, 192-345, 192-346, 192-347, 192-348, 192-349, 192-350, 192-351, 192-352, 192-353, 192-354, 192-355, 192-356, 192-357, 192-358, 192-359, 192-360, 192-361, 192-362, 192-363, 192-364, 192-365, 192-366, 192-367, 192-368, 192-369, 192-370, 192-371, 192-372, 192-373, 192-374, 192-375 or 192-376, or
- (c) 193-329, 193-330, 193-331, 193-332, 193-333, 193-334, 193-335, 193-336, 193-337, 193-338, 193-339, 193-340, 193-341, 193-342, 193-343, 193-344, 193-345, 193-346, 193-347, 193-348, 193-349, 193-350, 193-351, 193-352, 193-353, 193-354, 193-355, 193-356, 193-357, 193-358, 193-359, 193-360, 193-361, 193-362, 193-363, 193-364, 193-365, 193-366, 193-367, 193-368, 193-369, 193-370, 193-371, 193-372, 193-373, 193-374, 193-375 or 193-376, or
- (d) 230-271, 229-271, 229-272, 228-272, 228-273, ...*et seq*... 194-328, 194-329, or
a corresponding sequence of a chaperone polypeptide, said corresponding sequence sharing at least 50% homology with said amino acid sequence, wherein position 262 of said amino acid sequence or said corresponding sequence, is occupied by an amino acid residue other than alanine, and wherein position 267 of said amino acid sequence or said corresponding sequence, is occupied by an amino acid residue other than isoleucine.

13. (Currently Amended) A monomeric polypeptide, having chaperone activity and incapable of multimerisation, characterised in that, in the absence of ATP, the polypeptide has a protein refolding activity of more than 50%, said refolding activity being determined by contacting the polypeptide with an inactivated protein of known specific activity prior to inactivation, and then determining the specific activity of the said protein after contact with the polypeptide, the % refolding activity being:

$$\frac{\text{specific activity of protein after contact with polypeptide} \times 100}{\text{specific activity of protein prior to inactivation}}$$

wherein the selected amino acid sequence is selected from the group consisting of 230-271, 191-345, 191-376, 193-335 and 193-337 of GroEL (SEQ ID NO 10), ~~wherein positions 262 and 267~~

~~are occupied by amino acid residues other than alanine and isoleucine, respectively,~~ or a corresponding sequence of a chaperone polypeptide, said corresponding sequence sharing at least 50% homology with said amino acid sequence, wherein position 262 of said amino acid sequence or said corresponding sequence, is occupied by an amino acid residue other than alanine, and wherein position 267 of said amino acid sequence or said corresponding sequence, is occupied by an amino acid residue other than isoleucine.

14. (Previously Amended) A polypeptide as claimed in claim 1 further comprising a polyamino acid sequence, or an N-terminal polyamino acid sequence.

15. (Original) A polypeptide as claim in claim 14, wherein the polyamino acid sequence is a polyhistidine sequence.

16. (Previously Amended) A polypeptide as claimed in claim 14, wherein the polyamino acid sequence includes a cleavage site cleavable by a cleavage agent, or said cleavage agent is thrombin.

17. (Previously Amended) A polypeptide as claimed in claim 14 wherein the further polyamino acid sequence comprises a number of amino acid residues in the range 2 to 500, or 5 to 100, or 17 to 39.

18. (Previously Amended) A polypeptide as claimed in claim 1 in immobilized form, optionally immobilized to a chromatographic matrix, or an agarose resin.

19. (Original) A polypeptide as claimed in claim 18, wherein the agarose resin is a nickel-nitrilo-tri-acetic acid (NTA)-ligated agarose resin.

20. (Previously Amended) A polypeptide as claimed in claim 1 fused to a heterologous protein or polypeptide.

21. (Previously Amended) A recombinant polypeptide as claimed in claim 1.

22-28 (Cancelled)

29. (Previously Amended) A pharmaceutical formulation comprising a polypeptide of claim 1, optionally together with a diluent, carrier or excipient.

30. (Previously Amended) A polypeptide as defined in claim 1 for use in the treatment of disease.

31. (Previously Amended) The use of a polypeptide as defined in claim 1 in the manufacture of a medicament for the treatment of disease associated with protein/polypeptide structure.

32 – 54 (Cancelled)

55. (Currently Amended) A chaperone polypeptide having refolding activity and having an amino acid sequence selected from at least amino acid residues 230-271 but no more than residues 150-455 or 151-456 of a GroEL sequence as shown in Figure 7 (SEQ ID NO 10), ~~wherein position 262 is occupied by leucine and/or position 267 is occupied by methionine, or a~~ corresponding sequence of a chaperone polypeptide, said corresponding sequence sharing at least 50% homology with said amino acid sequence, wherein position 262 of said amino acid sequence or said corresponding sequence, is occupied by leucine, and/or position 267 of said amino acid sequence or said corresponding sequence, is occupied by methionine.

56. (Currently Amended) A monomeric polypeptide, having chaperone activity and incapable of multimerisation, characterised in that, in the absence of ATP, the polypeptide has a protein refolding activity of more than 50%, said refolding activity being determined by contacting the polypeptide with an inactivated protein of known specific activity prior to inactivation, and then determining the specific activity of the said protein after contact with the polypeptide, the % refolding activity being:

$$\frac{\text{specific activity of protein after contact with polypeptide}}{\text{specific activity of protein prior to inactivation}} \times 100$$

wherein the selected amino acid sequence is selected from the group consisting of 230-271, 191-345, 191-376, 193-335 and 193-337 of GroEL (SEQ ID NO 10), ~~wherein position 262 is occupied by leucine and/or position 267 is occupied by methionine~~, or a corresponding sequence of a chaperone polypeptide, said corresponding sequence sharing at least 50% homology with said amino acid sequence, wherein position 262 of said amino acid sequence or said corresponding sequence, is occupied by leucine, and/or position 267 of said amino acid sequence or said corresponding sequence, is occupied by methionine.

57. (New) A chaperone polypeptide having refolding activity and having an amino acid sequence selected from at least amino acid residues 230-271 but no more than residues 150-455 or 151-456 of a GroEL sequence as shown in Figure 7 (SEQ ID NO 10), wherein positions 262 and 267 are occupied by amino acid residues other than alanine and isoleucine, respectively.

58. (New) A polypeptide as claimed in claim 1 which comprises at least an amino acid sequence selected from GroEL residues:

- (a) 191-329, 191-330, 191-331, 191-332, 191-333, 191-334, 191-335, 191-336, 191-337, 191-338, 191-339, 191-340, 191-341, 191-342, 191-343, 191-344, 191-345, 191-346, 191-347, 191-348, 191-349, 191-350, 191-351, 191-352, 191-353, 191-354, 191-355, 191-356, 191-357, 191-358, 191-359, 191-360, 191-361, 191-362, 191-363, 191-364, 191-365, 191-366, 191-367, 191-368, 191-369, 191-370, 191-371, 191-372, 191-373, 191-374, 191-375 or 191-376, or
- (b) 192-329, 192-330, 192-331, 192-332, 192-333, 192-334, 192-335, 192-336, 192-337, 192-338, 192-339, 192-340, 192-341, 192-342, 192-343, 192-344, 192-345, 192-346, 192-347, 192-348, 192-349, 192-350, 192-351, 192-352, 192-353, 192-354, 192-355, 192-356, 192-357, 192-358, 192-359, 192-360, 192-361, 192-362, 192-363, 192-364, 192-365, 192-366, 192-367, 192-368, 192-369, 192-370, 192-371, 192-372, 192-373, 192-374, 192-375 or 192-376, or
- (c) 193-329, 193-330, 193-331, 193-332, 193-333, 193-334, 193-335, 193-336, 193-337, 193-338, 193-339, 193-340, 193-341, 193-342, 193-343, 193-344, 193-345, 193-346, 193-347, 193-348, 193-349, 193-350, 193-351, 193-352, 193-353, 193-354, 193-355, 193-356, 193-357, 193-358, 193-359, 193-360, 193-361, 193-362, 193-363, 193-364, 193-365, 193-366, 193-367, 193-368, 193-369, 193-370, 193-371, 193-372, 193-373, 193-374, 193-375 or 193-376, or

(d) 230-271, 229-271, 229-272, 228-272, 228-273, ...*et seq*... 194-328, 194-329.

59. (New) A monomeric polypeptide, having chaperone activity and incapable of multimerisation, characterised in that, in the absence of ATP, the polypeptide has a protein refolding activity of more than 50%, said refolding activity being determined by contacting the polypeptide with an inactivated protein of known specific activity prior to inactivation, and then determining the specific activity of the said protein after contact with the polypeptide. the % refolding activity being:

$$\frac{\text{specific activity of protein after contact with polypeptide} \times 100}{\text{specific activity of protein prior to inactivation}}$$

wherein the selected amino acid sequence is selected from the group consisting of 230-271, 191-345, 191-376, 193-335 and 193-337 of GroEL (SEQ ID NO 10), wherein positions 262 and 267 are occupied by amino acid residues other than alanine and isoleucine, respectively.

60. (New) A chaperone polypeptide having refolding activity and having an amino acid sequence selected from at least amino acid residues 230-271 but no more than residues 150-455 or 151-456 of a GroEL sequence as shown in Figure 7 (SEQ ID NO 10), wherein position 262 is occupied by leucine and/or position 267 is occupied by methionine.

61. (New) A monomeric polypeptide, having chaperone activity and incapable of multimerisation, characterised in that, in the absence of ATP, the polypeptide has a protein refolding activity of more than 50%, said refolding activity being determined by contacting the polypeptide with an inactivated protein of known specific activity prior to inactivation, and then determining the specific activity of the said protein after contact with the polypeptide, the % refolding activity being:

$$\frac{\text{specific activity of protein after contact with polypeptide} \times 100}{\text{specific activity of protein prior to inactivation}}$$

wherein the selected amino acid sequence is selected from the group consisting of 230-271, 191-345, 191-376, 193-335 and 193-337 of GroEL (SEQ ID NO 10), wherein position 262 is occupied by leucine and/or position 267 is occupied by methionine.